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A Cytological Marker Associated with Winterhardiness in Oat

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ABSTRACT

The intergenomic translocation T7C-17 occurs at different frequencies in fall- versus spring-sown hexaploid oat (*Avena* sp.) germplasm. The objectives of this experiment were to evaluate crown meristem freeze tolerance and winter field survival among 94 random F_4–derived lines from the cross between the cultivars Wintok (T7C-17, winterhardy) and Fulghum (non-T7C-17, less winterhardy) and to examine the association between these winterhardiness traits and T7C-17. Crown meristem freeze tolerance was evaluated in a three-replicate randomized complete block design in controlled environment growth cabinets. Field survival was evaluated in a three replicate randomized complete block design at Laurel Springs, NC during the 1999–2000 season. Greater crown meristem freeze tolerance and greater winter field survival were associated with the presence of T7C-17. Lines heterogeneous for the translocation had similar levels of crown meristem freeze tolerance and field survival as lines homozygous for the translocation. Twenty-two percent of the variation in crown meristem freeze tolerance and 27% of the variation in field survival was accounted for by translocation status. The observed frequencies of translocation homozygotes and heterozygotes did not fit the expected frequencies for single factor segregation in the F_4 generation. There were almost threefold as many homozygotes with the translocation as homozygotes without the translocation which indicated preferential selection for T7C-17 during inbreeding. Our results suggested that T7C-17 might be isolating, in terms of recombination, either a dominant allele or a group of loci conditioning winterhardiness in our population.

Reciprocal translocations have been observed in several studies of wild and cultivated hexaploid oat species (Ladizinsky, 1970; McMullen et al., 1982; Singh and Kolb, 1991; Wilson and McMullen, 1997). Rajhathy and Thomas (1974) suggested that such chromosomal rearrangements had a significant role in allopolyploid oat evolution and this hypothesis was supported by studies of the intergenomic translocation involving chromosomes 7C and 17 (T7C-17) (Zhou et al., 1999; Jellen and Beard, 2000). This particular reciprocal translocation has a long segment from chromosome 7C attached to chromosome 17, and a short segment from chromosome 17 attached to chromosome 7C. Zhou et al. (1999) found evidence for two independent paths of domestication from the hexaploid progenitor *A. sterilis* L. that were delineated by the presence or absence of T7C-17. Subsequently, Jellen and Beard (2000) observed that a preponderance of common oat (*A. sativa* L.) accessions contained T7C-17 in contrast to the absence of the translocation in most of red oat (*A. byzantina* C. Koch) accessions. They noted that 73% of accessions classified as spring habit had the T7C-17 karyotype while 69% of accessions classified as winter habit were homogeneous for the nontranslocation forms.

The prominent role played by T7C-17 in the delineation of *A. sativa* from *A. byzantina* germplasm and in the separation of fall- from spring-sown cultivars suggested that an investigation of the association of the translocation with differences in winterhardiness would be timely. The cultivars Fulghum and Wintok have been evaluated in the USDA-ARS coordinated Uniform Oat Winterhardiness Nursery since 1938. In side-by-side field comparisons in 487 combinations of locations and years, the mean winter survival of Fulghum was 35.9% and the mean winter survival of Wintok was 73.6% (Livingston and Elwinger, 1995) making these two cultivars ideal candidates for the proposed investigation.

The winter oat cultivar Fulghum originated as a late 19th century single plant selection from the landrace 'Red Rustproof' (Coffman, 1977). It does not contain T7C-17, which is consistent with its *A. byzantina* pedigree (Jellen and Beard, 2000). The winter oat cultivar Wintok was released in 1940 and both of its parents, 'Winter Fulghum' and 'Hairy Culberson', trace their pedigrees to the landrace Red Rustproof (Coffman, 1977). Winter Fulghum was a selection from Fulghum and Hairy Culberson was a selection from 'Culberson', which itself was a selection from Red Rustproof. Stanton (1955) cautioned that Culberson, from which Hairy Culberson was selected, might have been a mechanical seed mixture rather than a mutant in commercial seed of Red Rustproof. This possibility is lent credence by the fact that Wintok contains T7C-17, which is not consistent with it being a direct descendent of Red Rustproof (Jellen and Beard, 2000).

The specific objectives of this experiment were to evaluate crown meristem freeze tolerance and winter field survival among recombinant inbred lines from the cross between Fulghum (less winterhardy, non T7C-17) and Wintok (winterhardy, T7C-17), and to examine the association between these winterhardiness traits and T7C-17.

MATERIALS AND METHODS

Ninety-four random F_4–derived lines originating from independent F_2 plants from the cross between Fulghum and Wintok were evaluated in both controlled environment and field experiments. The cross was made in the winter of 1994–1995 and materials were advanced by single seed descent in the greenhouse. In each generation, pregerminated seedlings were vernalized in moist paper towels for 4 wk at 2°C before

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planting in pots on greenhouse benches. Data were not recorded on intergenerational survival of single seed descent lines.

The freezing tolerance of cold-hardened crowns was evaluated using a procedure similar to that described by Marshall et al. (1981). A randomized complete block design was utilized with three replications in time. Each replicate included 94 $F_{4:5}$ lines plus three entries of each parent. Seeds were planted 2 cm deep in 15-cm-long plastic nursery tubes filled with Metromix 220 (Scotts-Sierra Horticultural Products Co., Marysville, OH), one seed per tube and five tubes per entry. Tubes were placed in racks in a 9-m^2 walk-in controlled-environment chamber in the Southeastern Plant Environment Laboratory at North Carolina State University. Plants were grown for 5 wk in a regime of 12 h of light at a photosynthetic photon flux density of 225 μmol m^{-2} s^{-1}. A mixture of cool-white fluorescent (80% of input wattage) and incandescent (20% of input wattage) lamps provided the spectral energy between 400 and 700 nm. The temperature was 13°C and 10°C during the light and dark periods, respectively. The plants were transferred to a hardening chamber for 3 wk with a 12-h daylight intensity of 320 μmol m^{-2} s^{-1} and constant temperature of 2°C. Racks were randomly repositioned at weekly intervals in both chambers to eliminate variability due to position effects. Plants were watered three times per week with a solution containing a complete set of major and minor nutrients. Plants were watered with tap water on alternate days and weekends.

Plants were removed from tubes and washed in iced water after hardening. Crowns were prepared for freeze testing by partially trimming off roots and shoots. Approximately 3 to 4 cm of the stem base remained. Roots were trimmed to 0.5 cm from the base of the stem. Five crowns per entry were inserted into slits in circular moist sponges, placed in a plastic bag, set on an iron flange and placed in a freeze chamber at 2°C. Before sealing with a twist wire, 75 mL of crushed ice was placed in each bag to prevent supercooling. Temperature inside the freeze chamber was monitored using strategically located copper-constantin thermocouples. The crowns were subjected to second-phase cold hardening at −3°C for 48 h before the freeze test. Following this treatment the temperature was decreased 1°C per hour to −10°C and maintained at this temperature for 3 h. The temperature was then raised at 2°C per hour to 2°C and the crowns were allowed to thaw for 12 h. Their roots were trimmed and they were replanted in 50 by 30 cm boxes filled with Metromix to a depth of 5 cm and grown for 21 d at 13°C under controlled conditions. The five plants per entry were individually scored after 21 d using visual ratings on a scale of 0 (dead) to 5 (undamaged). The data underwent square root transformation (Gomez and Gomez, 1984) and were analyzed using the GLM procedure of the SAS software package (SAS Institute, 1999). The linear model utilized was a random effect randomized complete block design with sampling (Steel and Torrie, 1980).

Remnant seed of each of the 94 $F_{4:5}$ lines plus parents were planted at the Cunningham Research and Education Center, Kinston, NC, in late October 1998 to increase seed for field evaluations the following season. The 94 $F_{4:6}$ lines plus five entries of each of the two parents were planted in fall 1999 at two experiment stations in the Appalachian Mountains of North Carolina. Planting date at the Upper Mountain Research Station at Laurel Springs was 10 Sept. 1999. This station has an elevation of 895 m above sea level and mean winter temperature of 1.3°C. Planting date at the Mountain Research Station at Waynesville was 8 Oct. 1999. This station has an elevation of 727 m above sea level and a mean winter temperature of 5.3°C. The experimental design was a three replicate randomized complete block at both locations. Plots were single 1.83 m rows spaced 0.3 m apart with a seeding rate of 6 g per plot. Winter survival was recorded as percentage of plot regrowth in late March 2000. These data were adjusted according to germination rate for each plot that was recorded 1 mo after planting. Data analysis was performed using the GLM procedure of the SAS software package (SAS Institute, 1999). Heritabilities on a plot and entry-mean bases were calculated for crown meristem freeze tolerance and field survival using restricted maximum likelihood variance component and covariance matrix estimates generated by the mixed procedure in SAS (Holland et al., 2003).

Determination of the translocation status of each entry utilized $F_{4:6}$ seed. Root tips were collected from three germinating seedlings per line that had been pretreated as described in Lee et al. (1997) and Mitchell et al. (2003). Squashes and C-banding were performed using Giemsa stain as described in Jellen et al. (1993). The impact of translocation status on crown meristem freeze tolerance and field survival were determined using the GLM procedure of the SAS software package (SAS Institute, 1999). Differences in mean crown meristem freeze tolerance and field survival of lines with contrasting translocation status were compared using a paired *t* test.

RESULTS AND DISCUSSION

Crown Meristem Freeze Tolerance

Our results agreed with previous reports that controlled environment freeze tolerance in oat is a quantitative trait with moderate to high heritability (Amirshahi and Patterson, 1956; Muehlbauer et al., 1970). There was significant variation for freeze tolerance among the re-

Table 1. T7C-17 status, mean crown meristem freeze tolerance, and field survival at Laurel Springs, 2000 of parental and 94 recombinant inbred F_4–derived lines of winter oat.

Lines	No. of Samples	T7C-17 status	Crown meristem freeze tolerance	Transformed crown meristem freeze tolerance†	Field survival
	n		Rating 0–5‡		%
Fulghum	5	absent	0.06	0.74a§	25a
Wintok	5	present	1.93	1.46b	98b
F_4–derived	23	absent	1.06	1.20a	27a
F_4–derived	66	present	2.35	1.58b	68b
F_4–derived	5	heterogeneous	2.38	1.62b	68b
Heritability, % (plot basis)				30 ± 7	67 ± 5
Heritability, % (entry mean basis)				56 ± 8	85 ± 3
CV, %				34.9	33.0

† Square root transformation.
‡ Rating scale of 0 to 5, in which 0 = dead, and 5 = alive.
§ Means followed by same letter are not significantly different at 0.05 probability based on a *t* test.

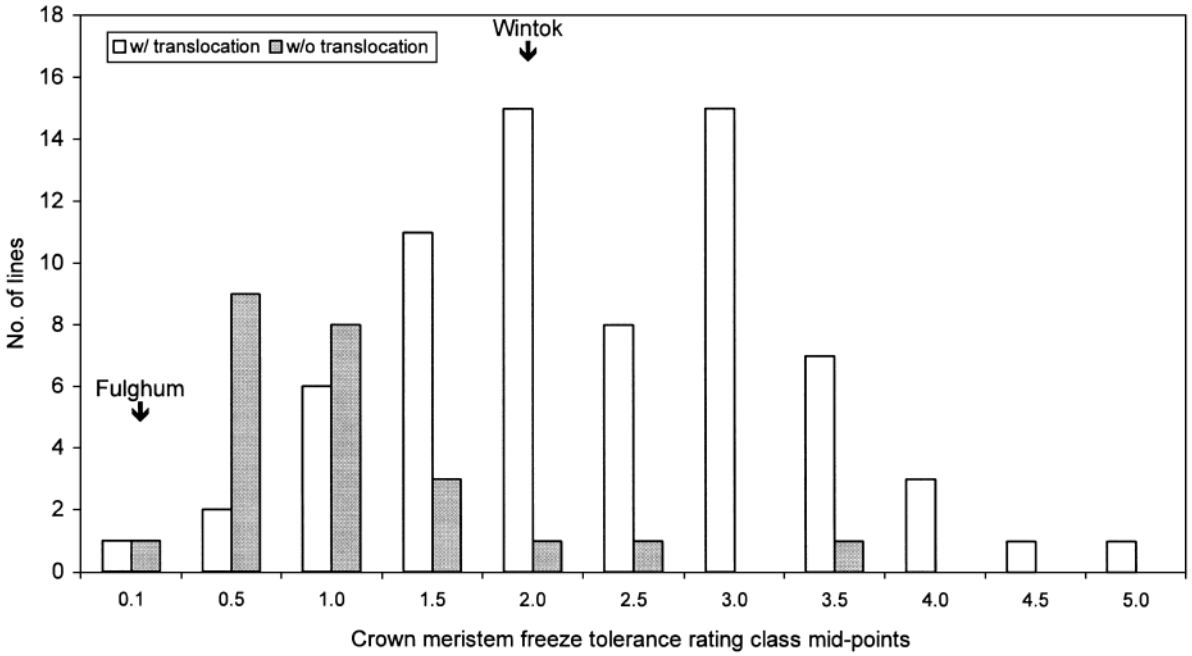

Fig. 1. Frequency distribution of crown meristem freeze tolerance ratings of 94 $F_{4:5}$ lines and their parents 'Fulghum' and 'Wintok' on a scale of 0 = dead to 5 = no freeze damage. Shading on colums representing each rating class indicates the number of lines without the T7C-17 translocation in that class.

combinant inbred lines (Table 1, Fig. 1). Variation within lines was not significant ($F = 1.93$, $P > 0.05$). Crown meristem freeze tolerance was normally distributed which is typical of a quantitative trait controlled by multiple loci. Heritability on a plot basis was 30 ± 7%, and heritability on an entry mean basis was 56 ± 8%. Coefficient of variation was similar to that reported by Muehlbauer et al. (1970).

The population mean crown meristem freeze tolerance was 2.0 with a range from 0.0 to 5.0. Wintok had significantly greater tolerance than Fulghum (1.9 versus 0.1), which was in agreement with previous controlled environment evaluations of these two cultivars (Livingston, 1996). A high frequency of positive transgressive segregates was observed. Nineteen of the 94 (20.2%) lines exceeded Wintok by greater than one $LSD_{(0.05)}$ indicating that the parents contained complementary alleles for this trait.

Field Survival

Significant variation among the recombinant inbred lines for field survival was observed at the Laurel Springs location only (Table 1, Fig. 2). Mean field survival was almost 100% at Waynesville and the data from this location were not utilized in subsequent analyses. Field survival at Laurel Springs tended toward a bimodal distribution with a population mean of 58% and a range of 2 to 100% (Fig. 2). Wintok had significantly greater field survival than Fulghum (98% versus 25%) which was in agreement with the multiyear data reported by Livingston and Elwinger (1995). Heritability on a plot basis was 67 ± 5%, which was lower than the mean of 79% reported by Muehlbauer et al. (1970) for 18 segregating populations evaluated in a severe winter in Pennsylvania. The coefficient of variation was similar to those reported in the annual Uniform Oat Winterhardiness Nursery (Livingston, unpublished data, 1926–2005). The Laurel Springs location was not effective in identification of positive transgressive segregates because Wintok had a mean field survival of 98%. Forty-seven lines were not significantly different from Wintok in field survival. There was a significant positive correlation of 0.47 ($P < 0.05$) between crown meristem freeze tolerance and field survival. Our correlation was less than the 0.81 reported by Marshall (1965), but our value was likely influenced by the limit in range of detectable field survival indicated by the 98% survival of Wintok.

Only one other report of a bimodal distribution was found in the literature. Amirshahi and Patterson (1956) noted that a normal distribution was typical for segregating progenies in 18 of 20 populations they evaluated in greenhouse tests. One bimodal distribution and one distribution skewed toward lower survival were found in the two remaining populations. Wintok was a parent in both populations with non normal distributions; however, four other populations with Wintok parentage were normally distributed.

The bimodal field survival distribution has two potential explanations. Fewer loci may be involved in the expression of field survival in that environment than crown meristem freeze tolerance. Alternately, several loci for different winterhardiness component traits may be linked to each other, or associated with T7C-17. Stacked alleles for winterhardiness component traits would be inherited similar to a major allele for winterhardiness causing a bimodal distribution. Winter-

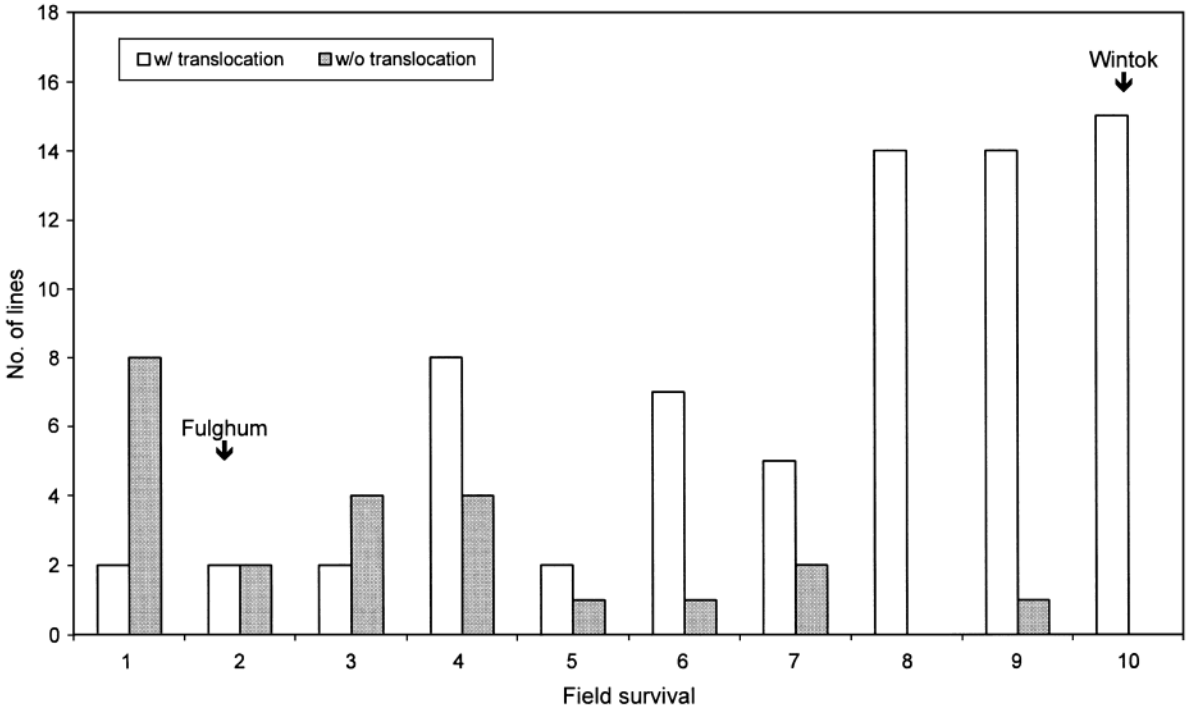

Fig. 2. Frequency distribution of field survival of 94 $F_{4:6}$ lines and their parents 'Fulghum' and 'Wintok' on a scale of 1 = 0–10% field survival, to 10 = 91–100% field survival. Shading on colums representing each survival class indicates the number of lines without the T7C-17 translocation in that class.

hardiness is a complex trait governed by a number of interacting components that vary in importance with environmental conditions (Thomashow, 1999). Several authors have reported linkage of winterhardiness component traits in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oat (Hayes et al., 1993; Galiba et al., 1995; Santos, 2000), further supporting the hypothesis of linked alleles.

Minimum daily temperatures of less than 10°C were recorded on nine dates at Laurel Springs, and the lowest temperature was −3°C on 28 Jan. 2000. Minimum daily temperatures of less than 10°C were recorded on 10 dates at Waynesville and the lowest temperature was −4°C on 27 and 28 Jan. 2000. The difference in field survival between the two locations reflected the absence of snow cover at the Laurel Springs test site when the minimum temperatures of late January were recorded. This illustrated the vagarious nature of field evaluation of winterhardiness and the reason controlled environment methodologies were developed (Marshall et al., 1981). It also underscored the importance of developing additional tools such as cytological or molecular markers linked to winterhardiness loci to aid selection.

7C-17 Translocation

T7C-17 was present in 66 (69%) and absent in 23 (24%) of the recombinant inbred lines (Table 1). Five (5%) lines were heterogeneous for the translocation. Only three plants per line were utilized to determine translocation status, thus the heterogeneous class frequency was likely underestimated. Nevertheless, there was almost threefold as many homozygotes with the translocation as there were homozygotes without the translocation among the F_4–derived progenies.

T7C-17 status was associated with crown meristem freeze tolerance and field survival (Table 1, Fig. 1,2). Progenies containing T7C-17 (Wintok type) had significantly higher crown meristem freeze tolerance and field survival scores than progenies that did not have T7C-17 (Fulghum type). Twenty-two percent of the variation in crown meristem freeze tolerance and 27% of the variation in field survival was accounted for by translocation status. Seventeen of the 25 lines with a crown meristem freeze tolerance rating less than 1.2 did not contain T7C-17. In contrast, 25 of the 26 lines with a freeze tolerance rating more than 2.8 contained the translocation. Sixteen of the 24 lines with a field survival less than 34% did not contain T7C-17, and 43 of the 44 lines with a field survival more than 70% did contain the translocation. Five lines were heterogeneous for T7C-17. The mean of the heterogeneous lines was equal to the mean of lines homozygous for the presence of T7C-17 for both winterhardiness variables (Table 1). This indicated dominance gene action at the winterhardiness locus or loci associated with the translocation.

The greater levels of winterhardiness in this population were associated with the presence of T7C-17. Although this was expected because the more winterhardy parent Wintok contained the translocation, winter oat germplasm and *A. byzantina* in particular have a low frequency of T7C-17 (Zhou et al., 1999; Jellen and Beard, 2000). Our results suggested that T7C-17 might be isolating, in terms of recombination, either a dominant allele or a group of loci conditioning winterhardiness in this population. Heterozygous translocations are known

to decrease recombination in the region around the breakpoints, presumably due to variable chromosome pairing (Bridges and Brehme, 1944) or lethality of gametes derived from recombination near the translocation (Burnham, 1962). The smaller the translocation segment the greater the chance that it will not pair with its homologous segment at pachytene and form a chain instead of a ring quadrivalent in the heterozygous nucleus (Burnham, 1962). With T7C-17, the translocation segment from 7C is long while the segment from 17 is so short as to hardly be noticeable on chromosome $7C^{17}$. O'Donoughue et al. (1995) and Wight et al. (2003) did not observe segregation distortion for markers associated with linkage group 3 in the 'Kanota' (non-T7C-17) by 'Ogle' (T7C-17) mapping population, in contrast to the findings of the present study, although the former reported distortion toward the Kanota alleles for a portion of linkage group 24 (chromosome 17). Groh et al. (2001) likewise did not find distortion for chromosome 7C-17 linkage groups in a second Kanota (non-T7C-17) by 'Marion' (T7C-17) population. Unfortunately, the recombinant inbred lines in those populations have not undergone cytogenetic analysis of their translocation status.

Although much of the original spring-sown North American germplasm was of northern European *A. sativa* origin and much of the original fall-sown North American germplasm was of Mediterranean *A. byzantina* origin, there were notable exceptions to this trend. The fall-sown *A. sativa* cultivar Winter Turf had significantly greater winterhardiness than Fulghum (Livingston and Elwinger, 1995). Winter Turf, which likely had a prominent role in 18th century U.S. winter oat production was an introduction from England (Coffman, 1977), contains T7C-17 and is found in the pedigrees of many winter oat cultivars. It would be interesting to follow T7C-17 from colonial germplasm through present day winter cultivars if DNA markers linked to key winterhardiness loci become available. One might determine if the Winter Turf T7C-17 and associated loci were inherited as a complex through successive breeding cycles.

No conscious selection was imposed on our population during development of inbred progenies, but the relative frequencies of the translocation classes indicated selection for T7C-17 during inbreeding in this population. Linkage with a gametic lethality factor or preferential transmission of translocation-containing gametes are possible explanations for this type of segregation distortion. Discrimination between these two possibilities without further molecular data is merely speculative at this point. However, it may be significant that T7C-17 was the predominant karyotypic form in the ancestral hexaploid oat, *A. sterilis*. It was present in approximately 80% of the accessions examined by Zhou et al. (1999) and these translocation genotypes were found throughout the range of the species. Further studies on the geographic range of T7C-17 in hexaploid *A. fatua* L., *A. hybrida* Peterm., *A. occidentalis* Dur., and *A. sterilis* ssp. *ludoviciana* (Durieu) Nyman revealed that the non-translocation karyotype is very rarely observed apart from *A. byzantina* and *A. sterilis* (Jellen et al., 2004). These studies also revealed the existence of a second, larger 7C-17 translocation in *A. fatua* accession CN 25955 from Morocco. In hindsight, the lack of data on intergenerational survival during the inbreeding process hampered the interpretation of our results. Such data should be routinely collected in studies with this translocation.

Our results have implications for cultivar development in populations segregating for T7C-17. The allelic content of a single or multilocus complex controlling winterhardiness associated with T7C-17 in Wintok is not necessarily the optimum. Siripoonwiwat et al. (1996), Holland et al. (1997), and Wooten et al. (2003) found QTL associated with heading date, vernalization response, and freeze tolerance on linkage groups assigned to both chromosomes 7C and 17 (Fox et al., 2001). Reduced recombination involving loci controlling components of winterhardiness would reduce genetic variation among progenies and necessitate evaluation of much larger populations than those routinely utilized to find rare, desirable recombinants in this region.

REFERENCES

Amirshahi, M.C., and F.L. Patterson. 1956. Cold resistance of parent varieties, F_2 populations, and F_3 lines of 20 oat crosses. Agron. J. 48:184–188.

Bridges, C.B., and K.S. Brehme. 1944. The mutants of *Drosophila melanogaster*. Carnegie Inst. of Washington Publ. 552, Washington, DC.

Burnham, C.R. 1962. Discussions in cytogenetics. C.R. Burnham, St. Paul, MN.

Coffman, F.A. 1977. Oat history, identification and classification. USDA-ARS Tech. Bull. No. 1516. U.S. Gov. Print. Office, Washington, DC.

Fox, S.L., E.N. Jellen, S.F. Kianian, H.W. Rines, and R.L. Phillips. 2001. Assignment of RFLP linkage groups to chromosomes using monosomic F1 analysis in hexaploid oat. Theor. Appl. Genet. 102: 320–326.

Galiba, G., S.A. Quarrie, J. Sutka, A. Morgounov, and J.W. Snape. 1995. RFLP mapping of the vernalization *(Vrn1)* and frost resistance *(Fr1)* genes on chromosome 5A of wheat. Theor. Appl. Genet. 90:1174–1179.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York.

Groh, S., A. Zacharias, S.F. Kianian, G.A. Penner, J. Chong, H.W. Rines, and R.L. Phillips. 2001. Comparative AFLP mapping in two hexaploid oat populations. Theor. Appl. Genet. 102:876–884.

Hayes, P.M., T.K. Blake, T.H.H. Chen, S. Tragoonrung, F. Chen, A. Pan, and B. Liu. 1993. Quantitative trait loci on barley (*Hordeum vulgare* L.) chromosome 7 associated with components of winter hardiness. Genome 36:66–71.

Holland, J.B., H.S. Moser, L.S. O'Donoughue, and M. Lee. 1997. QTLs and epistasis associated with vernalization responses in oat. Crop Sci. 37:1306–1316.

Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: An update. Plant Breed. Rev. 22:9–112.

Jellen, E.N., and J. Beard. 2000. Geographical distribution of a chromosome 7C and 17 intergenomic translocation in cultivated oat. Crop Sci. 40:256–263.

Jellen, E.N., D.E. Jarvis, D.C. Ames, I.G. Loskutov, F.D. Raymond, and P.J. Maughan. 2004. Genetic variation in the secondary and distant primary oat gene pools. *In* Abstracts of ASA Meeting, Seattle, WA. 1–4 Nov. 2004. ASA, Madison, WI.

Jellen, E.N., R.L. Phillips, and H.W. Rines. 1993. C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome 36:1129–1137.

Ladizinsky, G. 1970. Chromosome rearrangements in the hexaploid oats. Heredity 25:457–461.

Lee, J.H., K. Arumuganathan, Y. Yen, S. Kaeppler, H.F. Kaeppler, and P.S. Baenziger. 1997. Root tip cell cycle synchronization and

metaphase-chromosome isolation suitable for flow sorting in common wheat (*Triticum aestivum* L.). Genome 40:636–638.
Livingston, D.P., III. 1996. The second phase of cold hardening: Freezing tolerance and fructan isomer changes in winter cereal crowns. Crop Sci. 36:1568–1573.
Livingston, D.P., III, and G.E. Elwinger. 1995. Improvement of winter hardiness in oat from 1935 to 1992. Crop Sci. 35:749–755.
Marshall, H.G. 1965. A technique of freezing plant crowns to determine the cold resistance of winter oats. Crop Sci. 5:83–86.
Marshall, H.G., C.R. Olien, and E.H. Everson. 1981. Techniques for selection of cold hardiness in cereals. p. 139–158. *In* C.R. Olien and M.N. Smith (ed.) Analysis and improvement of plant cold hardiness. CRC Press, Boca Raton, FL.
McMullen, M.S., R.L. Phillips, and D.D. Stuthman. 1982. Meiotic irregularities in *Avena sativa* L. /*A. sterilis* L. hybrids and breeding implications. Crop Sci. 22:890–897.
Mitchell, C.C., S.E. Parkinson, T.J. Baker, and E.N. Jellen. 2003. C-banding and localization of 18S–5.8S–26S rDNA in tall oatgrass species. Crop Sci. 43:32–36.
Muehlbauer, F.J., H.G. Marshall, and R.R. Hill, Jr. 1970. Winterhardiness in oat populations derived from reciprocal crosses. Crop Sci. 10:646–649.
O'Donoughue, L.S., S.F. Kianian, P.J. Rayapati, G.A. Penner, M.E. Sorrells, S.D. Tanksley, R.L. Phillips, H.W. Rines, M. Lee, G. Fedak, S.J. Molnar, D. Hoffman, C.A. Salas, B. Wu, E. Autrique, and A. van Deynze. 1995. A molecular linkage map of cultivated oat. Genome 38:368–380.
Rajhathy, T., and H. Thomas. 1974. Cytogenetics of oats (*Avena* L.). Genet. Soc. Canada Misc. Publ. 2. Genet. Soc. Canada, Ottawa, ON.
Santos, A.G. 2000. Quantitative trait loci associated with components of winterhardiness in cultivated oat. Ph.D. diss. North Carolina State Univ., Raleigh, NC. (Diss. Abstr. Int. 61-07B:3330).
SAS Institute. 1999. The SAS system for Windows. Release 8.02. SAS Inst., Cary, NC.
Singh, R.J., and F.L. Kolb. 1991. Chromosomal interchanges in six hexaploid oat genotypes. Crop Sci. 31:726–729.
Siripoonwiwat, W., L.S. O'Donoughue, D. Wesenberg, D.L. Hoffman, J.F. Barbosa-Neto, and M.E. Sorrells. 1996. Chromosomal regions associated with quantitative traits in oat [Online]. Available at www.cabi-publishing.org/ jag/papers96/paper396/indexp396.html (verified 12 Nov. 2005). J. Quant. Trait Loci 2:Article 3.
Stanton, T.R. 1955. Oat identification and classification. USDA-ARS Tech. Bull. No. 1100. U.S. Gov. Print. Office, Washington, DC.
Steel, R.J., and J.H. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill Book Company, NY.
Thomashow, M.F. 1999. Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:571–599.
Wight, C. P., N.A. Tinker, S.F. Kianian, M.E. Sorrells, L.S. O'Donoughue, D.L. Hoffman, S. Groh, G.L. Scoles, ChengDao Li, F.H. Webster, R.L. Phillips, H.W. Rines, S.M. Livingston, K.C. Armstrong, G. Fedak, and S.J. Molnar. 2003. A molecular marker map in 'Kanota' × 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46:28–47.
Wilson, W.A., and M.S. McMullen. 1997. Recombination between a crown rust resistance locus and an interchange breakpoint in hexaploid oat. Crop Sci. 37:1694–1698.
Wooten, D.R., D.P. Livingston, J.P. Murphy, and J.B. Holland. 2003. Freeze tolerance QTL in a winter oat by spring oat recombinant inbred population. *In* Annual meetings abstracts [CD-ROM]. ASA, Madison, WI.
Zhou, X., E.N. Jellen, and J.P. Murphy. 1999. Progenitor germplasm of domesticated hexaploid oat. Crop Sci. 39:1208–1214.